IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of
Makoto SUNAGAWA et al.
Serial No. 10/550,395 Group Art Unit: 1624
Filed: September 22, 2005 Examiner: BERCH, Mark L.
For: NOVEL CARBAPENEM COMPOUNDS

DECLARATION

Honorable Commissioner of
Patents and Trademarks
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Katsunori Kanazawa, a citizen of Japan, declare and say as follows.

1. I am a graduate of Master Course at the Department of Microbiology, Graduate School of Kyoto Pharmaceutical University, Japan in March 1992 and received Doctor of Philosophy at Faculty of Pharmaceutical Sciences, Tokushima Bunri University in 2006.

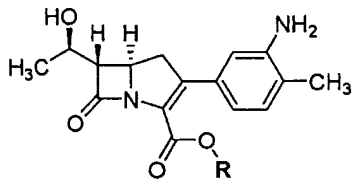
2. Since April 1992 up till the present, I have been an employee of Dainippon Sumitomo Pharma Co., Ltd. (former name: Sumitomo Pharmaceuticals Company, Limited), the assignee of U.S. Patent Application No. 10/550,395, and I have been engaged in developmental research works with respect to new antibiotics at the Research Center of said company.

3. I am familiar with the subject matter of U.S. Patent Application No. 10/550,395.

4. Under my direction, the following experiments have been done.

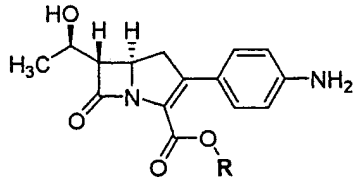
Test Compounds:

The following compounds were subjected to Experiments 1 and 2.



Compound 1a
= **R** : H

Compound 1b
= **R** : CH_2OCO-tBu



Compound 2a
= **R** : H

Compound 2b
= **R** : CH_2OCO-tBu

Compounds 1a and 1b are compounds disclosed in Example 47 and Example 48 of the present specification.

Compound 2a is a compound substituted CH_2NH_2 with NH_2 in Guthikonda's compound disclosed in Guthikonda and compound 2b is a compound prepared by esterification of carboxyl group at position 2 of compound 2a with pivaloyloxymethyl iodide.

Experiment 1: Antibacterial Activities of Carbapenem Compounds

With respect to carbapenem compound 1a of the present invention and reference compound 2a, the antibacterial activities against various microorganisms were tested as follows.

i) Test methods:

The antibacterial activities were tested by an agar dilution method according to the standard method defined by Japanese Society of Chemotherapy [Chemotherapy, vol.129, p76-79 (1981)] as follows.

The test compound was dissolved in distilled water, which was diluted stepwise, and the diluted solution of the test compound was mixed into an agar medium to give a test compound-containing agar plate wherein the

test compound was contained in the final concentration so as to be two-fold dilution series.

Mueller Hinton Agar medium (MHA) was used. The test bacterial strains on agar plate were cultured in an incubator at 37°C overnight. Each strain thus cultured was suspended in Buffered Saline with Gelatin (BSG) in a concentration of about 10^6CFU/ml, and the suspension of strain (about 5µl) was inoculated onto the test compound-containing agar medium using a microplanter. The agar medium was cultured at 37°C for about 20 hours. Then, the growth of microorganisms was observed. The minimum concentration (MIC, µg/ml) of the test compound was defined as the lowest drug concentration that completely prevented visible growth of the organism tested.

ii) Test results:

Strains		Compound 1a	Compound 2a
S. epidermidis	IAM1296	0.125*	0.25
E. faecalis	ATCC19433	0.25	0.25
E. faecium	ATCC19434	1	1
K. pneumoniae	ATCC10031	0.031	0.063
P. mirabiris	GN2425	0.125	0.25
P. vulgaris	OX-19	0.125	0.25
S. marcescens	x100	0.125	0.125
E. aerogenes	ATCC13048	2	1
S. aureus	MS9408	0.5	0.5
E. coli	ML1410	0.125	0.125
E. coli	ML1410RP4	0.125	0.125
E. cloacae	GN7471	2	2
P. vulgaris	GN7919	0.125	0.25
S. marcescens	GN6473	2	1
PRSP*	MIC_{50}	0.063	0.063
	MIC_{90}	0.125	0.125
BLNAR*	MIC_{50}	0.125	0.5
	MIC_{90}	0.25	0.5

* : MIC(μg/ml)

PRSP: Penicillin resistant *Streptcoccus pneumoniae*

BLNAR: β-lactamase non-producing ampicillin-resistant *Haemophilus influenzae*

iii) Consideration:

As shown in the above table, compound 1a of present invention shows the almost same as or slightly better in antibacterial activity against various bacteria comparing with reference compound 2a.

Experiment 2: Oral Absorbability of Carbapenem Compounds

With respect to carbapenem compound 1b of the present invention and reference compound 2b, the oral absorbability was tested as follows.

i) Test method for oral absorbability in rats:

Male SD rats (7 weeks old) (n=3) were used. The rats were fastened since preceding day but were freely given with pure water only.

The test compound (1mg/mL, conversion as the free compound) was dissolved in a 5% DMSO - 0.5% methylcellulose mixture. Cilastatin (100mg/kg) was subcutaneously administered to the rats. After 5 minutes, the test compound was orally administered in an amount of 10mL/kg (=10mg/kg, conversion as the free compound). At 5, 15, 30 and 60 minutes after administration of the test compound, the rats were bled, and the blood thus obtained was centrifuged to separate blood serum. The concentration of the test compound in the blood serum was measured by bio-assay using *Bacillus subtilis* ATCC6633 as an indicator bacteria.

Separately, the test compound (the free compound, 5mg/mL, 2mL/kg=10mg/kg) was dissolved in saline solution containing 25mM 3-morpholino propane sulfonic acid (MOPS), and the solution was administered into caudal vein of the rats, and the blood serum was obtained and subjected to the bioassay, likewise.

ii) Measurement of bioavailability:

The concentrations of the test compound in blood serum obtained above in oral administration were drawn in a graph per time after administration, and the area under the curve (the curve of the blood serum concentration of the test compound – time) (AUC) was calculated. The AUC when the test compound was administered into the caudal vein was also calculated likewise. The absolute bioavailability (BA) was calculated by the following equation:

BA(%) = (AUC in oral administration / AUC in intravenous administration) x 100

iii) Test results:

	Compound 1b	Compound 2b
Bioavailability (%)	26	4

iv) Consideration:

As shown in the above table, compound 1b of present invention shows more than 6 times as much as in oral bioavailability comparing with reference compound 2b. This means that compound 1a shows in vivo antibacterial activities more than 6 times as much as compound 2b.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and brief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 28th day of April, 2008

Katsunori Kanazawa

Katsunori Kanazawa